

CLAIMS

What is claimed is:

1. An isolated polynucleotide encoding an outer membrane protein of *E. canis*, a variant of said outer membrane protein, or an antigenic fragment of said protein; wherein the outer membrane protein is selected from the group consisting of P30, P30a, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10, P30-11, and P30-12.
2. The isolated polynucleotide of claim 1 wherein said polynucleotide encodes an amino acid sequence which is at least 95% identical to a sequence selected from the group consisting of:
 - amino acid 26 through amino acid 288 of the sequence, SEQ ID NO: 32, shown in Figure 19B;
 - amino acid 26 through amino acid 287 of the sequence, SEQ ID NO: 34, shown in Figure 20B,
 - amino acid 55 through amino acid 307 of the sequence, SEQ ID NO: 36, shown in Figure 21B,
 - amino acid 26 through amino acid 280 of the sequence, SEQ ID NO: 38, shown in Figure 22B,
 - amino acid 26 through amino acid 283 of the sequence, SEQ ID NO: 40, shown in Figure 23B,
 - amino acid 26 through amino acid 276 of the sequence, SEQ ID NO: 42, shown in Figure 24B,
 - amino acid 27 through amino acid 293 of the sequence, SEQ ID NO: 44, shown in Figure 25B,
 - amino acid 31 through amino acid 293 of the sequence, SEQ ID NO: 54, shown in Figure 26B,
 - amino acid 31 through amino acid 296 of the sequence, SEQ ID NO: 56, shown in Figure 27B,
 - amino acid 27 through amino acid 299 of the sequence, SEQ ID NO: 46, shown in Figure 28B,
 - amino acid 27 through amino acid 281 of the sequence, SEQ ID NO: 58, shown in Figure 29B,
 - amino acid 26 through amino acid 280 of the sequence, SEQ ID NO: 48, shown in Figure 30B,
 - amino acid 26 through amino acid 279 of the sequence, SEQ ID NO: 60, shown in Figure 31B,
 - amino acid 1 through amino acid 241 of the sequence, SEQ ID NO: 62, shown in Figure 32B.
3. The isolated polynucleotide of claim 1 wherein said polynucleotide encodes the P30 protein, a variant of the P30 protein or an antigenic fragment of said P30 protein.
4. The isolated polynucleotide of claim 1 wherein said polynucleotide encodes a sequence which is at least 95% identical to a sequence comprising amino acid 33 through amino acid 224 of the sequence, SEQ ID NO: 32, shown in Figure 19B.
5. The isolated polynucleotide of claim 3 wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of the coding sequence shown in FIG

19A, FIG 20A, FIG 21 A, FIG 22A, FIG 23A, FIG 24 A, FIG 25A, FIG 26A, FIG 27A, FIG 28A, FIG 29A, FIG 30A, FIG 31A, and FIG 32B.

6. An isolated polynucleotide encoding an outer membrane protein of *E. chaffeensis*, a variant of said outer membrane protein, or an antigenic fragment of said outer membrane protein, wherein the outer membrane protein is selected from the group consisting of OMP-1, OMP-1A, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y, OMP-1Z, and OMP-1H.

Fig 9A 1B

7. The isolated polynucleotide of claim 6 wherein the polynucleotide encodes an amino acid sequence which is at least 95% identical to a sequence selected from the group consisting of:

amino acid 26 through amino acid 281 of the sequence, SEQ ID NO 2, shown in Figure 3B;

amino acid 29 through amino acid 196 of the sequence, SEQ ID NO 16, shown in Figure 10B,

amino acid 26 through amino acid 291 of the sequence, SEQ ID NO 18, shown in Figure 11B,

amino acid 1 through amino acid 131 of the sequence, SEQ ID NO 20, shown in Figure 12B,

amino acid 26 through amino acid 295 of the sequence, SEQ ID NO 22, shown in Figure 13B,

amino acid 27 through amino acid 279 of the sequence, SEQ ID NO 24, shown in Figure 14B,

amino acid 30 through amino acid 283 of the sequence, SEQ ID NO 26, shown in Figure 15B,

amino acid 25 through amino acid 275 of the sequence, SEQ ID NO 28, shown in Figure 16B,

amino acid 28 through amino acid 285 of the sequence, SEQ ID NO 30, shown in Figure 17B,

amino acid 27 through amino acid 300 of the sequence, SEQ ID NO 50, shown in Figure 18B;

amino acid 27 through amino acid 298 of the sequence, SEQ ID NO 52, shown in Figure 33B.

8. The isolated polynucleotide of claim 6 wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of the coding sequence set forth in FIG 3A, FIG 10A, FIG 11A, FIG 12A, FIG 13 A, FIG 14A, FIG 15A, FIG 16 A, FIG 17A, FIG 18A, and Fig 33A.

9A

9. An isolated polypeptide selected from the group consisting of the P30 protein, a variant of the P30 protein, an antigenic fragment of the P30 protein, the P30a protein, a variant of the P30a protein, the P30-1 protein, a variant of the p30-1 protein, the P30-2 protein, a variant of the P30-2 protein, the P30-3 protein, a variant of the P30-3 protein, the P30-4 protein, a variant of the P30-4 protein, the P30-5 protein, a variant of the P30-5 protein, the P30-6 protein, a variant of the P30-6 protein, the P30-7 protein, a variant of the P30-7 protein, the P30-8 protein, a

variant of the P30-8 protein, the P30-9 protein, a variant of the P30-9 protein, the P30-10 protein, a variant of the P30-10 protein, a P30-11 protein, a variant of the P30-11 protein, the P20-12 protein, and a variant of the P30-12 protein.

10. The isolated polypeptide of claim 9 wherein said polypeptide comprises a sequence which is at least 95% identical to a sequence selected from the group consisting of:

of:
 amino acid 26 through amino acid 288 of the sequence, SEQ ID NO: 32, shown in Figure 19B;
 amino acid 26 through amino acid 287 of the sequence, SEQ ID NO: 34, shown in Figure 20B,
 amino acid 55 through amino acid 307 of the sequence, SEQ ID NO: 36, shown in Figure 21B,
 amino acid 26 through amino acid 280 of the sequence, SEQ ID NO: 38, shown in Figure 22B,
 amino acid 26 through amino acid 283 of the sequence, SEQ ID NO: 40, shown in Figure 23B,
 amino acid 26 through amino acid 276 of the sequence, SEQ ID NO: 42, shown in Figure 24B,
 amino acid 27 through amino acid 293 of the sequence, SEQ ID NO: 44, shown in Figure 25B,
 amino acid 31 through amino acid 293 of the sequence, SEQ ID NO: 54, shown in Figure 26B,
 amino acid 31 through amino acid 296 of the sequence, SEQ ID NO: 56, shown in Figure 27B,
 amino acid 27 through amino acid 299 of the sequence, SEQ ID NO: 46, shown in Figure 28B,
 amino acid 27 through amino acid 281 of the sequence, SEQ ID NO: 58, shown in Figure 29B,
 amino acid 26 through amino acid 280 of the sequence, SEQ ID NO: 48, shown in Figure 30B,
 amino acid 26 through amino acid 279 of the sequence, SEQ ID NO: 60, shown in Figure 31B,
 amino acid 1 through amino acid 241 of the sequence, SEQ ID NO: 62, shown in Figure 32B.

11. The isolated polypeptide of claim 9 wherein said polypeptide is the P30 protein, a variant of the P30 protein, or an antigenic fragment of the P30 protein.

12. The isolated polypeptide of claim 9 wherein said polypeptide comprises a sequence which is at least 95% identical to a sequence comprising amino acid 33 through amino acid 224 of the sequence, SEQ ID NO: 32, shown in Figure 19B.

13. An isolated polypeptide selected from the group consisting of the OMP-1 protein, the OMP-1R protein, a variant of the OMP-1R protein, the OMP-1S protein, a variant of the OMP-1S protein, the OMP-1T protein, a variant of the OMP-1T protein, the OMP-1U protein, a variant of the OMP-1U protein, the OMP-1V protein, a variant of the OMP-1V protein, the OMP-1W protein, a variant of the OMP-1W protein, the OMP-1X protein, a variant of the OMP-1X protein,

the OMP-1Y protein, a variant of the OMP-1Y protein, the OMP-1Z protein, a variant of the OMP-1Z protein, the OMP-1H protein, a variant of the OMP-1H protein.

14. The polypeptide of claim 3 wherein said polypeptide comprises a sequence which is at least 95% identical to a sequence selected from the group consisting of:

amino acid 26 through amino acid 281 of the sequence, SEQ ID NO 2, shown in Figure 3B;
 amino acid 29 through amino acid 196 of the sequence, SEQ ID NO 16, shown in Figure 10B, ← 9B
 amino acid 26 through amino acid 291 of the sequence, SEQ ID NO 18, shown in Figure 11B,
 amino acid 1 through amino acid 131 of the sequence, SEQ ID NO 20, shown in Figure 12B,
 amino acid 26 through amino acid 295 of the sequence, SEQ ID NO 22, shown in Figure 13B,
 amino acid 27 through amino acid 279 of the sequence, SEQ ID NO 24, shown in Figure 14B,
 amino acid 30 through amino acid 283 of the sequence, SEQ ID NO 26, shown in Figure 15B,
 amino acid 25 through amino acid 275 of the sequence, SEQ ID NO 28, shown in Figure 16B,
 amino acid 28 through amino acid 285 of the sequence, SEQ ID NO 30, shown in Figure 17B,
 amino acid 27 through amino acid 300 of the sequence, SEQ ID NO 50, shown in Figure 18B,
 amino acid 27 through amino acid 298 of the sequence, SEQ ID NO 52, shown in Figure 33B.

15. A method for diagnosing an infection with *E. chaffeensis* in a patient comprising the steps of:

(a) providing a serum sample from the patient;
 (b) providing a polypeptide selected from the group consisting of the polypeptide of claim 9, the polypeptide of claim 3, and mixtures thereof;
 (c) contacting the serum sample with the polypeptide; and
 (d) assaying for the formation of a complex between antibodies in the serum sample and the polypeptide, wherein formation of said complex is indicative of infection with *E. chaffeensis*.

16. The method of claim 5 wherein said polypeptide is the P30 protein, a variant of the P 30 protein, or an antigenic fragment of the P30 protein.

17. The method of claim 6 wherein the polypeptide has an amino acid sequence which is at least 95% identical to amino acid 33 through amino acid 224 of the sequence, SEQ ID NO:32, shown in Fig. 19B.

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18. The method of claim 6 wherein said polypeptide has an amino acid sequence comprising amino acid 26 through amino acid 281 of the sequence, SEQ ID NO:2, shown in FIG 3B.

19. A method for diagnosing an infection with *E. canis* in a Canidae patient comprising the steps of:

- (a) providing a serum sample from the patient ;
- (b) providing a polypeptide of claim 9;
- (c) contacting the serum sample with the outer membrane protein; and
- (d) assaying for the formation of a complex between antibodies in the serum sample and the polypeptide, wherein formation of said complex is indicative of infection with *E. canis*.

20. An antibody which binds to a protein selected from the group consisting of P30, P30a, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10, P30-11, P30-12, OMP-1, OMP-1A, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y, OMP-1Z, OMP-1H and combinations thereof.

21. A kit for diagnosing *E. chaffeensis* in a patient, said kit comprising a reagent selected from the group consisting of: the polypeptide of claim 3, the P30 protein, a variant of the P30 protein, an antigenic fragment of the P30 protein, and combinations thereof.

22. The kit of claim 19 wherein further comprising a biomolecule for detecting interactions between the reagent and antibodies in a bodily sample of the patient.

23. An immunogenic composition comprising a polypeptide of claim 9 or a polypeptide of claim 11 and a pharmaceutically acceptable adjuvant.

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